Bioorganic & Medicinal Chemistry Letters 22 (2012) 6486–6489

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Allylic thiocyanates as a new class of antitubercular agents

Gustavo P. Silveira [a], Misael Ferreira [b], Luciano Fernandes [b,†], Garrett C. Moraski [c], Sanghyun Cho [d], Changhwa Hwang [d], Scott G. Franzblau [d], Marcus M. Sá [b,*]

[a] Departamento de Química, Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS 91501-970, Brazil
[b] Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil
[c] Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA
[d] Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

ARTICLE INFO

Article history:
Received 14 May 2012
Revised 31 July 2012
Accepted 13 August 2012
Available online 21 August 2012

Keywords:
Anti-tuberculosis agents
Morita–Baylis–Hillman
Allylic azide
Allylic thiocyanate
Isothiouronium salt

ABSTRACT

TB is a global public health emergency in which new drugs are desperately needed. Herein we report on the synthesis of a diverse panel of 41 aryl allylic azides, thiocyanates, isothiouronium salts, and *N*,*N*′-diacetylisothioureas that were evaluated for their in vitro activity against replicating and non-replicating *Mycobacterium tuberculosis* (*Mtb*) $H_{37}Rv$ and toxicity to VERO cells. We found a selective group of new and promising compounds having good (micromolar) to excellent (sub-micromolar) potency against replicating *Mtb* $H_{37}Rv$. Allylic thiocyanates bearing halophenyl (halo = 2-Br, 4-Br, 4-Cl, 4-F), 4-methylphenyl and 2-naphthyl moieties were the most active as antitubercular agents. In particular, the 2-bromophenyl-substituted thiocyanate showed MIC = 0.25 μM against replicating *Mtb*, MIC = 8.0 μM against non-replicating *Mtb* and IC_{50} = 32 μM in the VERO cellular toxicity assay.

Tuberculosis (TB) is caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*). This dreadful disease has been responsible for the deaths of over one billion people and currently infects one-third of the world's population.[1] In 1993, when an estimated 7–8 million cases and 1.3–1.6 million deaths were occurring, the World Health Organization (WHO) declared TB a global public health emergency. In 2010, there were still an estimated 8.5–9.2 million cases and 1.2–1.5 million deaths (including deaths from TB among HIV-positive people)[1] indicating improvements have been made but the battle is far from won. The spread of TB was temporarily interrupted by DOTS (directly observed treatment, short course) of isoniazid, rifampicin, ethambutol and pyrazinamide in combination.[2] However, the increase of TB/HIV co-infection continues to grow among people living with HIV, accounting for about 13% of TB cases registered in 2010.[1] Therefore, there is an urgent need to develop new antitubercular drugs to combat the spread of this virulent disease.[3]

Our laboratory has had a long research interest in the Morita–Baylis–Hillman (MBH) reaction,[4–7] resulting in the preparation of various allylic azides, thiocyanates, isothiouronium salts, and *N*,*N*′-diacetylisothioureas in aqueous medium as versatile scaffolds for synthesis (Scheme 1). The biological activity related to the thiocyanate functionality is sparsely documented.[8,9] On the other hand, isothiouronium salts have attracted the attention of the scientific community due to their unique and wide spread chemical and biological properties.[10] They have been explored as agonists of GABA-type receptors,[11] as prodrugs of the aldehyde dehydrogenase inhibitor cyanamide,[12] as local anesthetics,[13] and as antibacterial agents.[14] While the structurally related thiosemicarbazones are known to possess pronounced antituberculosis activity associated with low cytotoxicity,[15] much less attention has been devoted to investigate the screening of isothiouronium salts and analogs against *Mtb*.[16]

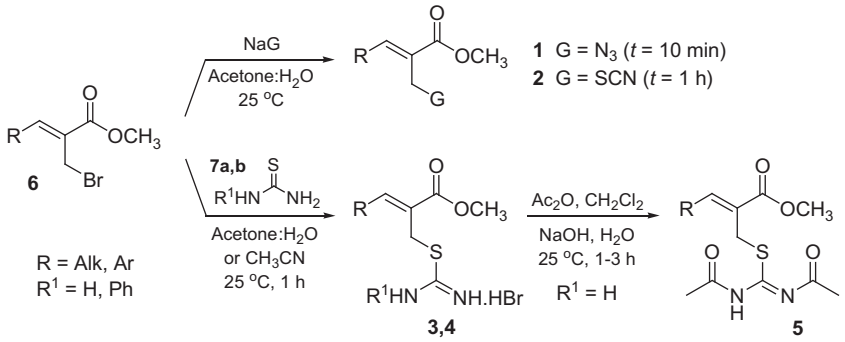
Herein we present our preliminary results involving the screening of aryl allylic azides **1**, thiocyanates **2**, isothiouronium salts **3** and **4**, and *N*,*N*′-diacetylisothioureas **5** (Scheme 1) against *Mtb* $H_{37}Rv$ for the development of possible new antitubercular agents.

The key allylic bromides **6** participate in halide displacement by nucleophiles in aqueous medium to give different classes of substituted allylic derivatives in good to high yields according to previous reports[5–7,17] (Scheme 1). Therefore, allylic bromides **6**, which are readily available in multigram scale by treating α-methylene-β-hydroxyesters (MBH adducts)[18–20] with $LiBr/H_2SO_4$ in acetonitrile at room temperature,[21] were the common precursors for azides **1**, thiocyanates **2,** and isothiouronium salts **3** and **4** depending on the nucleophile employed.

The synthesis of allylic azides **1** was performed by reacting bromides **6** with sodium azide in aqueous acetone at room temperature for 10–15 min.[7,17,22] Similarly, allylic thiocyanates **2** were

* Corresponding author. Tel.: +55 48 37216844; fax: +55 48 37216850.
E-mail address: marcus.sa@ufsc.br (M.M. Sá).
† Present address: Coordenação de Engenharia Química, Universidade Tecnológica Federal do Paraná, Ponta Grossa, PR 84016-210, Brazil.

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.08.048



Scheme 1. Synthetic routes for the preparation of allylic azides **1**, thiocyanates **2**, isothiouronium salts **3** and **4**, and *N*,*N*′-diacetylisothioureas **5** from bromides **6**.

obtained by mixing the bromide **6** with NaSCN in the same conditions,[23] but in these cases a longer period (1 h) was required for the reaction to attain >95% conversion to the expected product.[6] All azides **1** and thiocyanates **2** were stable compounds readily isolated in high yields after flash chromatography.

Isothiouronium salts **3** were also readily prepared from **6** by halide displacement in aqueous acetone, in this case employing thiourea (**7a**) as an ambident nucleophile[5] to give salts **3** as crystalline solids. The synthesis of *N*-phenyl-substituted isothiouronium salts **4** from bromides **6** and phenylthiourea (**7b**) was also achieved with excellent chemoselectivity, providing that aqueous acetone is replaced by acetonitrile as the reaction solvent. Under these conditions, *S*-alkylated salts **4** were formed as crystalline solids that were readily collected by filtration without any further purification. Considering that either the sulfur or one of the nitrogen atoms in **7b** is able to participate in a nucleophilic attack on one of the several electrophilic sites available in the allylic bromides **6**, the selective formation of isothiouronium salt **4** as the sole product in high yields is noteworthy.

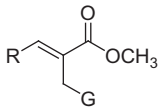
Finally, the *N*,*N*′-diacetyl isothioureas **5** were obtained by acetylation of the corresponding salts **3** in aqueous alkaline medium[5] (Scheme 1) in up to 3 h to give the diacetylated products as crystalline solids after recrystallization.

MICs against replicating *Mtb* $H_{37}Rv$ were determined by the microplate Alamar Blue assay (MABA).[24] MICs against non-replicating (NR) *Mtb* were determined using the low-oxygen-recovery assay (LORA).[25] Toxicity was measured against VERO cells[26] and reported as the half maximal inhibitory concentration (IC_{50}). A total of 41 derivatives were synthesized for this study and evaluated for their in vitro antitubercular activity and toxicity. There is about a twofold error within any whole-cell screen and we take this into consideration as we describe the SAR trends presented in Table 1. We spent considerable synthetic effort to develop an understanding of the structure–activity relationship (SAR) of the each scaffold (and the analogs generated) within this whole-cell screening paradigm.

Most of the allylic azides **1** were inactive against *Mtb* (MIC > 128 μM). The only exception was the azide **1a** bearing a 4-nitrophenyl group (MIC = 4.0 μM). It is known that DprE1, a mycobacterial nitroreductase involved in the cell-wall biogenesis, can be inhibited by some aromatic nitro compounds through a FAD-dependent mechanism where the nitro group is reduced to the corresponding nitroso function.[27] This process might be related to the moderate activity observed for the nitro-substituted allylic azide **1a**.

However, allylic thiocyanates **2** exhibited an opposite trend in comparison with azides **1**. The 4-nitrophenyl thiocyanate **2a** was inactive (MIC > 128 μM) whilst its 2-substituted isomer **2b** and analogs bearing electron-releasing groups (**2c**–**f**) were also poorly active. Gratifyingly, thiocyanates substituted with weakly-donating groups (**2h,i**), as well as most of the halo-substituted derivatives (**2j,m**–**o**) showed very potent inhibition against *Mtb* in sub-micromolar MIC range (Table 1).

Table 1
Potency of allylic azides **1**, thiocyanates **2**, isothiouronium salts **3** and **4**, and *N*,*N*′-diacetylisothioureas **5** against replicating *Mtb* (MIC in μM) in GAS medium[a,b]



#	G	R	MIC (μM)
1a	N_3	4-$NO_2C_6H_4$	4.0
1b	N_3	2-$NO_2C_6H_4$	64
1c	N_3	(*E*)-$C_6H_5CH{=}CH$	64
1d	N_3	3,4-$(OCH_2O)C_6H_3$	64
1e	N_3	4-$CH_3OC_6H_4$	>128
1f	N_3	C_6H_5	>128
1g	N_3	2-$C_{10}H_7$	>128
1h	N_3	4-ClC_6H_4	>128
1i	N_3	2-ClC_6H_4	>128
1j	N_3	2,4-$Cl_2C_6H_3$	>128
2a	SCN	4-$NO_2C_6H_4$	>128
2b	SCN	2-$NO_2C_6H_4$	8.0
2c	SCN	(*E*)-$C_6H_5CH{=}CH$	>128
2d	SCN	3,4-$(OCH_2O)C_6H_3$	>128
2e	SCN	4-$CH_3OC_6H_4$	16
2f	SCN	3,4-$(CH_3O)_2C_6H_3$	16
2g	SCN	C_6H_5	1.0
2h	SCN	2-$C_{10}H_7$	0.25
2i	SCN	4-$CH_3C_6H_4$	0.50
2j	SCN	4-ClC_6H_4	0.25
2k	SCN	2-ClC_6H_4	>128
2l	SCN	2,4-$Cl_2C_6H_3$	>128
2m	SCN	2-BrC_6H_4	0.25
2n	SCN	4-BrC_6H_4	0.25
2o	SCN	4-FC_6H_4	0.50
3a	SC(NH)NH_2·HBr	4-$NO_2C_6H_4$	8.0
3b	SC(NH)NH_2·HBr	3,4-$(OCH_2O)C_6H_3$	>128
3c	SC(NH)NH_2·HBr	4-$CH_3OC_6H_4$	32
3d	SC(NH)NH_2·HBr	C_6H_5	64
3e	SC(NH)NH_2·HBr	2-ClC_6H_4	>128
4a	SC(NC_6H_5)NH_2·HBr	4-$NO_2C_6H_4$	4.0
4b	SC(NC_6H_5)NH_2·HBr	3-$NO_2C_6H_4$	16
4c	SC(NC_6H_5)NH_2·HBr	2-$NO_2C_6H_4$	16
4d	SC(NC_6H_5)NH_2·HBr	3,4-$(OCH_2O)C_6H_3$	16
4e	SC(NC_6H_5)NH_2·HBr	4-$CH_3OC_6H_4$	16
4f	SC(NC_6H_5)NH_2·HBr	C_6H_5	16
4g	SC(NC_6H_5)NH_2·HBr	4-$CH_3C_6H_4$	16
4h	SC(NC_6H_5)NH_2·HBr	4-ClC_6H_4	8.0
4i	SC(NC_6H_5)NH_2·HBr	4-FC_6H_4	8.0
5a	SC($NCOCH_3$)$NHCOCH_3$	C_6H_5	32
5b	SC($NCOCH_3$)$NHCOCH_3$	2-ClC_6H_4	32

[a] Values reported are the average of three individual measurements and rounded to nearest dilution.
[b] GAS = glycerol-alanine-salts medium.

In the case of the 2-naphthyl-derived thiocyanate **2h** (MIC = 0.25 μM) and the 4-methylphenyl analog **2i** (MIC = 0.50 μM), their slightly higher potency compared with that observed for the unsubstituted phenyl thiocyanate **2g** (MIC = 1.0 μM) suggests the hypothesis that having a tethered hydrophobic group on the aromatic ring is beneficial to the activity. The excellent activity of both 2- and 4-bromo-substituted allylic thiocyanates **2m** and **2n** (MIC = 0.25 μM) corroborates the existence of favorable hydrophobic effects. Conversely, substitution with the more electronegative chlorine at the 2-position (**2k** and **2l**) led to a dramatic loss in the activity against *Mtb* (MIC > 128 μM). However, the introduction of a chlorine solely on the 4-position (as in the derivative **2j**) still maintained the inhibition at sub-micromolar figures (MIC = 0.25 μM). The 4-fluoro substituted thiocyanate **2o** also showed a high level of *Mtb* inhibition (MIC = 0.50 μM), which is probably related to the stereoelectronic effects that are particularly prominent in governing the behavior of fluorinated organic compounds.[28]

Because of the much more pronounced activity against *Mtb* observed for the allylic thiocyanates **2** compared with azides **1**, we turned our attention on other allylic scaffolds that contain variants of the S–C–N moiety. Therefore, representative isothiouronium salts and acetylated derivatives were screened against replicating *Mtb* (Table 1).

In all but one case (**3a**, MIC = 8.0 μM), the allylic isothiouronium salts **3** were only modestly effective against *Mtb* (MIC's of 32–128 μM), while the *N*-phenyl-substituted isothiouronium salts **4a**–**j** presented homogeneously good activity (MIC's of 4.0–16 μM) for the nine compounds tested. Nevertheless, none compared favorably with the results observed for the allylic thiocyanates **2**, in particular, the isothiouronium salts **4** substituted with haloaryl groups (F and Cl) were significantly less potent than the corresponding thiocyanate analogs **2**.

Acetylation of both amino groups of the allylic isothioureido moiety of **3** did not bring any advantage in terms of improving the activity against *Mtb* (Table 1, compounds **5a** and **5b,** MIC = 32 μM).

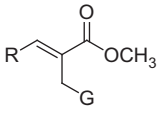
Antibacterial agents are often prioritized on the basis of their in vitro activity as well as other parameters. However, the apparent inhibitory activity of new leads can be misleading because most culture media do not reproduce an environment relevant to infection in vivo. Glycerol has been known to be the preferred source of energy and carbon for *M. tuberculosis* under in vitro conditions. As such, glycerol is present in most standard *M. tuberculosis* growth media.[26]

To further validate the potency of the allylic derivatives studied, the most potent compounds were additionally screened in a culture medium (7H12) which lacks glycerol.[25] The 7H12 medium contains palmitic acid as its carbon source while the GAS medium consists of glycerol–alanine–salts. Selected results are displayed in Table 2. It is noteworthy that compounds **2h**–**j** and **2m**–**o** all decreased in activity in the glycerol-free medium (MIC's in the range of 8.0–32 μM). This is suggestive of possible carbon source dependence[29] or some other effect related to the differences in the culture medium.

Of greater importance, however, are the good activities (MIC's from 4.0–16 μM) observed in the LORA assay, which uses the 7H12 medium under low oxygen conditions (Table 2). The LORA assay was specifically designed to simulate non-replicating *M. tuberculosis* during persistence and/or latency. Given the limited number of compounds that have shown micromolar potency in this assay,[30] the results achieved with the promising class of allylic thiocyanates **2** are certainly encouraging.

For the selected compounds tested, VERO screening indicated low toxicity values with reasonable selectivity indices in most cases,[31] showing a VERO IC_{50}/*Mtb* MIC ratio higher than 100 for the more promising compounds **2h**, **2m** and **2n** (Table 2).

Table 2
Anti-TB activity and selectivity of the best leads



#	G	R	MIC (μM) versus *Mtb*		IC_{50} (μM)
			Replicating[a]	NR[b]	VERO
1a	N_3	4-$NO_2C_6H_4$	>32	16	>64
2h	SCN	2-$C_{10}H_7$	32	16	32
2i	SCN	4-$CH_3C_6H_4$	8	16	32
2j	SCN	4-ClC_6H_4	8	8	16
2m	SCN	2-BrC_6H_4	8	4	32
2n	SCN	4-BrC_6H_4	16	16	32
2o	SCN	4-FC_6H_4	16	16	32
3a	IB[c]	4-$NO_2C_6H_4$	64	NT	NT
4a	PIB[d]	4-$NO_2C_6H_4$	>32	>64	>64

[a] Cultured in Middlebrook 7H12 medium.
[b] NR = non-replicating *Mtb* using the Low oxygen recover assay (LORA). VERO = African green monkey kidney cell line. NT = not tested. Values reported are the average of three individual measurements and rounded to nearest dilution.
[c] IB = isothiouronium bromide [$SC(NH)NH_2$·HBr].
[d] PIB = *N*-phenylisothiouronium bromide [$SC(NC_6H_5)NH_2$·HBr].

In conclusion, our laboratories identified various small allylic thiocyanates and isothiouronium salts that are active against *Mtb*. We reported five new and promising compounds having good (micromolar) to excellent (sub-micromolar) potency against replicating *Mtb* H_{37}Rv. Two of the most potent compounds (**2j** and **2m**) also displayed moderate activity against non-replicating (NR) *Mtb* in the low oxygen recovery assay with MICs of 8.0 and 4.0 μM, respectively. Additionally, evaluation in the VERO cellular toxicity assay revealed a good selectivity index in some cases, particularly with the two potent compounds **2j** and **2m** (IC_{50} of 16 and 32 μM, respectively). We are currently synthesizing and screening other halogenated thiocyanates as well as conformationally constrained and unconstrained derivatives in order to gain more information associated with this new class of antitubercular agents, and the results will be reported in due course.

Acknowledgments

This research was supported in part by Grant 2R01AI054193 from the National Institutes of Health (NIH). The excellent technical assistance of Baojie Wan and Yuehong Wang with anti-TB assays at UIC is greatly appreciated. The authors wish to thank the Central de Análises (Departamento de Química, UFSC) for spectroscopic analysis. G.P.S. thanks FAPERGS for supporting the short training in microbiology assays, and to Jed Fisher for regular and lasting scientific discussions. L.F. and M.F. are grateful to CNPq and CAPES (Brazilian Research Councils) for fellowships. M.M.S. is grateful to CNPq for a research fellowship. Financial support from INCT-Catalysis/CNPq is also gratefully acknowledged.

Supplementary data

Supplementary data (copies of ^1H and ^{13}C NMR spectra for the novel compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.048.

References and notes

1. (a) Global tuberculosis control WHO report 2011. WHO/HTM/TB/2011.16.; (b) Kwan, C. K.; Ernst, J. *Clin. Microbiol. Rev.* **2011**, *24*, 351.
2. Snider, D. E., Jr.; Raviglione, M.; Kochi, A. *Global Burden of Tuberculosis, Tuberculosis: Pathogenesis; Protection, and Control*; ASM Press: Washington, D.C., 1994.

3. (a) Lamichhane, G. *Trends Mol. Med.* **2011**, *17*, 25; (b) Nathan, C. *Cell Host Microbe.* **2009**, *5*, 220; (c) Carvalho, L. P. S.; Lin, G.; Jiang, X.; Nathan, C. *J. Med. Chem.* **2009**, *52*, 5789; (d) West, N. P.; Cergol, K. M.; Xue, M.; Randall, E. J.; Britton, W. J.; Payne, R. J. *Chem. Commun.* **2011**, *47*, 5166; (e) Russell, D. G.; Barry, C. E., 3rd; Flynn, J. L. *Science* **2010**, *328*, 852.
4. Meier, L.; Ferreira, M.; Sá, M. M. *Heteroatom Chem.* **2012**, *23*, 179.
5. Sá, M. M.; Ferreira, M.; Bortoluzzi, A. J.; Fernandes, L.; Cunha, S. *Arkivoc* **2010**, *xi*, 303.
6. Sá, M. M.; Fernandes, L.; Ferreira, M.; Bortoluzzi, A. J. *Tetrahedron Lett.* **2008**, *49*, 1228.
7. Sá, M. M.; Ramos, M. D.; Fernandes, L. *Tetrahedron* **2006**, *62*, 11652.
8. Liñares, G. G.; Gismondi, S.; Codesido, N. O.; Moreno, S. N. J.; Docampo, R.; Rodriguez, J. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5068.
9. Capon, R. J.; Skene, C.; Liu, E. H.-T.; Lacey, E.; Gill, J. H.; Heiland, K.; Friedel, T. *J. Nat. Prod.* **2004**, *67*, 1277.
10. Zhang, G.; Jin, W.; Fukushima, T.; Kosaka, A.; Ishii, N.; Aida, T. *J. Am. Chem. Soc.* **2007**, *129*, 719.
11. Allan, R. D.; Dickenson, H. W.; Johnston, G. A. R.; Kazlauskas, R.; Mewett, K. N. *Neurochem. Int.* **1997**, *30*, 583.
12. Shirota, F. N.; Stevens-Johnk, J. N.; DeMaster, E. G.; Nagasawa, H. T. *J. Med. Chem.* **1997**, *40*, 1870.
13. Regan, B. M.; Galysh, F. T.; Morris, R. N. *J. Med. Chem.* **1967**, *10*, 649.
14. Iwai, N.; Fujii, T.; Nagura, H.; Wachi, M.; Kitazume, T. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 246.
15. Pavan, F. R.; Maia, P. I. S.; Leite, S. R. A.; Deflon, V. M.; Batista, A. A.; Sato, D. N.; Franzblau, S. G.; Leite, C. Q. F. *Eur. Med. Chem.* **2010**, *45*, 1898.
16. Glasser, A. C.; Doughty, R. M. *J. Pharm. Sci.* **1965**, *54*, 1055.
17. Sá, M. M. *J. Braz. Chem. Soc.* **2003**, *14*, 1005.
18. Basavaiah, D.; Reddy, B. S.; Badsara, S. S. *Chem. Rev.* **2010**, *110*, 5447.
19. Basavaiah, D.; Rao, A. J.; Satyanarayana, T. *Chem. Rev.* **2003**, *103*, 811.
20. Singh, V.; Batra, S. *Tetrahedron* **2008**, *64*, 4511.
21. Ferreira, M.; Fernandes, L.; Sá, M. M. *J. Braz. Chem. Soc.* **2009**, *20*, 564.
22. Foucaud, A.; Guemmout, F. E. *Bull. Soc. Chim. Fr.* **1989**, 403.
23. *Typical procedure for the synthesis of allylic thiocyanates* **2**. To a stirred solution of allylic bromide **6** (1.0 mmol) in 4.0 mL of acetone/H_2O (3:1 v/v) at 25 °C was added 2.0 mmol of NaSCN. After stirring for 1 h, the final mixture was diluted with CH_2Cl_2 and washed with H_2O and brine. The organic extract was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by chromatography (hexane/ethyl acetate 9:1) to give the corresponding (*Z*)-2-(thiocyanomethyl)alkenoates **2**. Spectral and analytical data for selected compounds are as follows:
*Methyl (Z)-3-(4-chlorophenyl)-2-(thiocyanomethyl)-2-propenoate (**2j**).* Yield 85%; white solid, mp 71.3–71.8 °C. IR (KBr): ν_{max}/cm^{-1} 3045, 2951, 2149, 1705, 1620, 1584, 1435, 1274, 1199. ^1H NMR (400 MHz, $CDCl_3$): δ 3.89 (s, 3H), 4.09 (s, 2H), 7.37 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.1, 52.8, 111.8, 126.4, 129.4, 130.6, 132.1, 136.1, 143.5, 166.2.
*Methyl (Z)-3-(2-bromophenyl)-2-(thiocyanomethyl)-2-propenoate (**2m**).* Yield 97%; white solid, mp 72.0–73.5 °C. IR (KBr): ν_{max}/cm^{-1} 3060, 2952, 2153, 1717, 1635, 1436, 1289, 1088. ^1H NMR (400 MHz, $CDCl_3$): δ 3.90 (s, 3H), 3.93 (s, 2H), 7.25–7.29 (m, 1H), 7.37–7.44 (m, 2H), 7.64 (d, J = 7.8 Hz, 1H), 7.96 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.2, 52.9, 112.0, 123.9, 127.8, 128.0, 129.9, 131.0, 133.2, 134.3, 143.7, 165.9.
*Methyl (Z)-3-(4-bromophenyl)-2-(thiocyanomethyl)-2-propenoate (**2n**).* Yield 87%; white solid, mp 71.5–72.5 °C. IR (KBr): ν_{max}/cm^{-1} 3062, 2949, 2150, 1714, 1622, 1586, 1488, 1435, 1275, 1159. ^1H NMR (400 MHz, $CDCl_3$): δ 3.89 (s, 3H), 4.07 (s, 2H), 7.29 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.91 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.1, 52.9, 111.8, 124.4, 126.6, 130.8, 132.4, 132.6, 143.6, 166.2.
24. Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
25. Cho, S. H.; Warit, S.; Wan, B.; Hwang, C. H.; Pauli, G. F.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **2007**, *51*, 1380.
26. Falzari, K.; Zhu, Z.; Pan, D.; Liu, H.; Hongmanee, P.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **2005**, *49*, 1447.
27. Trefzer, C.; Skovierová, H.; Buroni, S.; Bobovská, A.; Nenci, S.; Molteni, E.; Pojer, F.; Pasca, M. R.; Makarov, V.; Cole, S. T.; Riccardi, G.; Mikusová, K.; Johnsson, K. *J. Am. Chem. Soc.* **2012**, *134*, 912.
28. Zimmer, L. E.; Sparr, C.; Gilmour, R. *Angew. Chem., Int. Ed.* **2011**, *50*, 11860.
29. Pethe, K.; Sequeira, P. C.; Agarwalla, S.; Rhee, K.; Kuhen, K.; Phong, W. Y.; Patel, V.; Beer, D.; Walker, J. R.; Duraiswamy, J.; Jiricek, J.; Keller, T. H.; Chatterjee, A.; Tan, M. P.; Ujjini, M.; Rao, S. P. S.; Camacho, L.; Bifani, P.; Mak, P. A.; Ma, I.; Barnes, S. W.; Chen, Z.; Plouffe, D.; Thayalan, P.; Ng, S. H.; Au, M.; Lee, B. H.; Tan, B. H.; Ravindran, S.; Nanjundappa, M.; Lin, X.; Goh, A.; Lakshminarayana, S. B.; Shoen, C.; Cynamon, M.; Kreiswirth, B.; Dartois, V.; Peters, E. C.; Glynne, R.; Brenner, S.; Dick, T. *Nat. Commun.* **2010**, *1*, 57.
30. Moraski, G. C.; Markley, L. D.; Chang, M.; Cho, S.; Franzblau, S. G.; Hwang, C. H.; Boshoff, H.; Miller, M. *J. Bioorg. Med. Chem.* **2012**, *20*, 2214.
31. TAACF (Tuberculosis Antimicrobial Acquisition and Coordinating Facility)—Global discovery program for novel anti-tuberculosis drugs. Available: http://www.taacf.org/data-interpretation.htm#cytotoxdata [accessed July 27, 2012].